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FEBS Letters 339 (1994) 213–216

FEBS 13662

FEBS LETTERS

The rapid and reversible association of phosphofructokinase with myocardial membranes during myocardial ischemia

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Received 20 December 1993; accepted 11 January 1994

Abstract

Myocardial calcium-independent phospholipase A_2 (PLA_2) activity is mediated by a 400 kDa catalytic complex comprised of a tetramer of phosphofructokinase (PFK) and a 40 kDa catalytic subunit [1,2]. During myocardial ischemia, calcium-independent PLA_2 activity rapidly and reversibly translocates from the cytosol to a membrane-associated compartment where it has been implicated as a mediator of ischemic damage [3,4]. Herein we demonstrate that the majority of both PFK mass and activity is translocated from the cytosol to a membrane-associated compartment prior to the onset of irreversible myocytic injury and that translocated PFK is catalytically inactive while membrane-associated. Furthermore, reperfusion of ischemic myocardium, or treatment of membranes derived from ischemic myocardium with ATP results in the conversion of both PFK mass and activity from its membrane-associated state to a soluble, catalytically-competent form. Collectively, these studies demonstrate that the concomitant changes in glycolysis and phospholipid hydrolysis during early myocardial ischemia result, at least in part, from the translocation of a common regulatory polypeptide critical in both processes.

Key words: Phosphofructokinase; Ischemia; Phospholipase A_2; Myocardium

1. Introduction

Although alterations in glycolytic flux during the evolution of myocardial ischemia are multifactorial in nature, the allosteric modulation of phosphofructokinase (PFK) activity by P_i and ATP is the predominant mechanism underlying the regulation of glycolysis during early myocardial ischemia [5–9]. Previous work has demonstrated the close temporal association between the onset of myocardial ischemia, increases in glycolytic flux and accelerated phospholipid hydrolysis [10–14]. Substantial evidence has now been accrued implicating the activation and translocation of a calcium-independent PLA_2 as the enzymic mechanism responsible for the accelerated hydrolysis of critical myocardial membrane constituents which results in ischemic membrane dysfunction [3,4]. In myocardium, calcium-independent PLA_2 activity is catalyzed by a 400 kDa catalytic complex comprised of a tetrameric PFK-like regulatory polypeptide and a 40 kDa catalytic element [1,2,15]. The demonstration that a common polypeptide (i.e. PFK) is critical to both glycolysis and phospholipolysis has suggested that a unifying molecular mechanism may be responsible for the coordinate changes in glycolytic flux and phospholipid hydrolysis during early myocardial ischemia [1].

Several studies have indirectly demonstrated that some glycolytic enzymes translocate to membrane-associated subcellular compartments during the evolution of the ischemic process, but definitive evidence demonstrating alterations in membrane-associated protein mass of specific glycolytic constituents has not yet been presented [8,16–18]. To investigate the temporal course of the translocation of PFK during the evolution of ischemic injury, we utilized an immunoaffinity purified, monospecific antibody directed against PFK to identify ischemia-induced alterations in the subcellular compartmentation of PFK mass and activity. We now report that PFK, the enzyme mediating the rate-determining step in anaerobic glycolysis, undergoes a rapid and reversible translocation to a membrane fraction during myocardial ischemia where it remains catalytically inactive until it is released by reperfusion of ischemic myocardium.

2. Materials and methods

2.1. Langendorff perfusion of rabbit hearts

Control and ischemic rabbit hearts were perfused retrograde through the aorta in the Langendorff mode as previously described [3,4]. After a 10 min pre-equilibration interval, the hearts were rendered globally ischemic (zero flow), perfused continuously, or, following a 15 min global ischemic episode, reperfused for the indicated times. Perfusions were terminated by the excision of ventricular myocardium which was rapidly homogenized in 10 mM Tris-HCl, 2 mM EDTA, 1 mM DTT, pH 8.0 at 4°C utilizing a polytron apparatus. The supernatant and

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SSDI 0014-5793(94)00046-X

particulate fractions were separated by centrifugation at 24,000 × g_{max} for 20 min as previously described [8]. The resultant 24,000 × g_{max} pellet was resuspended in buffer (10 mM Tris-HCl, 50 mM $MgSO_4$, 5 mM DTT, 0.5 mM ATP, pH 8.0) at a concentration of 0.3 mg/ml.

2.2. Immunological techniques

Chicken IgY affinity-purified monospecific antibody directed against rabbit skeletal muscle phosphofructokinase was prepared as previously described [1]. Western blots were performed utilizing 10% SDS-polyacrylamide gels employing established procedures [1,19]. To facilitate comparisons of the mass of PFK in the soluble and particulate fractions, microsomes were resuspended in a volume of homogenization buffer equal to that from which they were derived so that the intensity of the 85 kDa immunoreactive band in cytosol and microsomes is directly comparable to the amount of total mass present in each fraction.

2.3. Miscellaneous procedures and source of materials

PFK activity was measured using a spectrophotometric assay as previously described [20]. A Bio-Rad protein assay kit was used to measure protein content according to the manufacturer's instructions. Most reagents were purchased from Sigma.

3. Results

3.1. Translocation of phosphofructokinase during myocardial schemia

In control hearts, the overwhelming majority of PFK mass and activity was present in the cytosolic compartment (Fig. 1). After only 5 min of myocardial ischemia, translocation of phosphofructokinase from the cytosolic to a membrane-associated fraction was manifest (Fig. 1). After 15 min of ischemia, the majority of PFK mass was present in a membrane-associated fraction which was accompanied, pari passu, by a decrease in the mass of PFK immunoreactive material in the cytosolic fraction. Although large amounts of PFK immunoreactive material were present in the membrane-associated fraction, it was catalytically incompetent until released by ATP (Fig. 2). Since irreversible cellular injury is not manifest until after 30 min of acute myocardial ischemia in this model (vide infra), these results demonstrate that the translocation of PFK from the cytosolic fraction to a membrane-associated fraction precedes myocytic cellular necrosis.

Reversibility is an essential component of biologically important regulatory processes. Accordingly, we examined the reversibility of the association of PFK with the membrane-associated fraction after reperfusion of globally ischemic myocardium. In independent experiments, the majority of PFK immunoreactive material in control myocardium was again present in the soluble fraction, while that present after 15 min of myocardial ischemia was predominantly membrane-associated (lanes 1 and 2, Fig. 3). Reperfusion of myocardium rendered ischemic for 15 min resulted in the translocation of PFK in the particulate fraction to the cytosolic compartment (lanes 3 and 4, Fig. 3). Collectively, these results demonstrate the rapid and reversible translocation of PFK from myocardial cytosol to a membrane-associated fraction where it is catalytically incompetent until released by reperfusion.

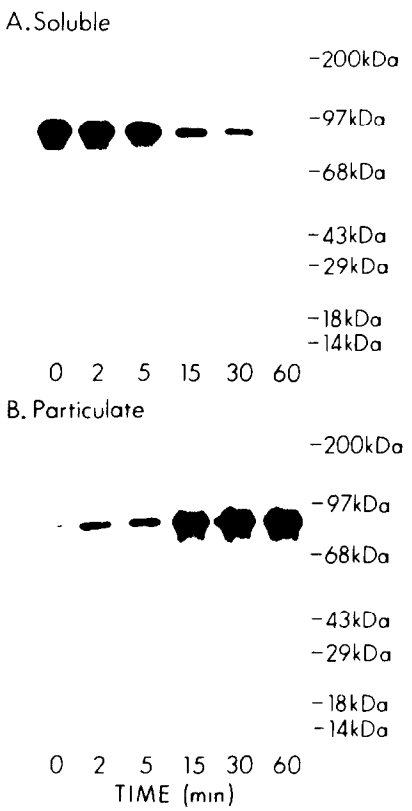

Fig. 1. Rabbit hearts were perfused retrograde in the Langendorff mode as described in section 2. After a 10 min equilibration period, the hearts were rendered globally ischemic for 0 (control), 2, 5, 15, 30 or 60 min (panels A and B) prior to preparation of the 24,000 × g_{max} supernatants and pellets for analysis by Western blot with affinity-purified anti-PFK antibody. To facilitate direct comparisons, pellets from centrifugation were resuspended in a volume of homogenization buffer equal to that from which they were derived as described in section 2. A single 85 kDa immunoreactive band was present. The majority of the immunoreactive PFK mass was present in the 24,000 × g_{max} supernatant (panel A; 0, 2, 5 min) which decreased in intensity with increasing durations of ischemia (panel A; 15, 30, 60 min). The 85 kDa immunoreactive band was present in low amounts in the 24,000 × g_{max} pellet at early time points (panel B; 0, 2, 5 min) and increased with the duration of ischemia (panel B; 15, 30, 60 min).

4. Discussion

Although the precise biochemical mechanisms mediating the alterations of glycolytic flux during anaerobic metabolism are not known in exact molecular detail, the possibility that glycolytic enzymes associate as a complex and translocate to membrane-associated subcellular fractions and contribute to the modulation of glycolytic flux has received substantial attention [6–8,16–18]. For example, 'delay-dissected' (i.e. 1 h postmortem) sheep heart PFK activity is associated with the particulate fraction, while PFK activity in fresh sheep hearts is present in the soluble fraction [8]. Aldolase also undergoes a similar subcellular redistribution in perfused rat hearts during ischemia and/or anoxia [7].

Recently, we have shown that myocardial calcium-independent PLA_2 activity is mediated by a 400 kDa

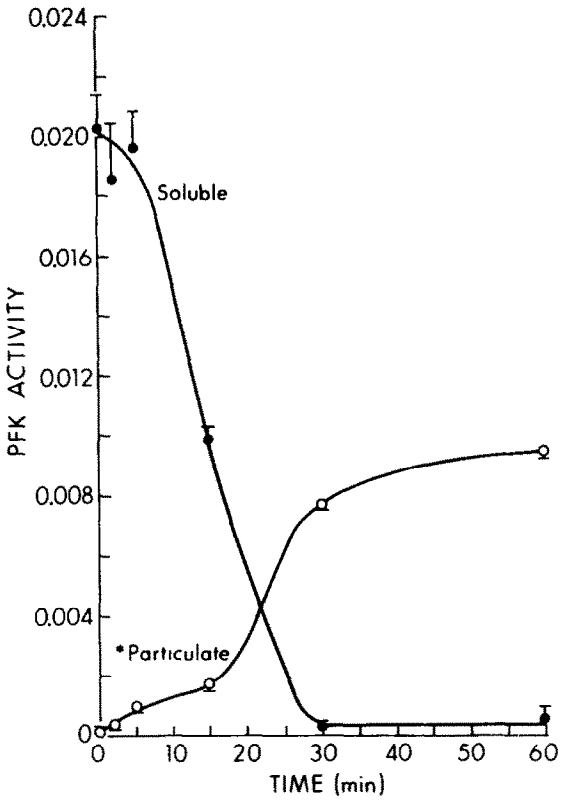

Fig. 2. PFK activity assays were performed spectrophotometrically utilizing either the 24,000 × g_{max} supernatant or resuspended pellets obtained after homogenization of perfused hearts as described in Fig. 1 (0 (control) or 2, 5, 15, 30, 60 min of ischemia). Soluble PFK activity (supernatant) decreased between 5 and 15 min, and reached a minimum (i.e. no measurable activity) at 30 min. PFK activity in the pellet (assessed after its desorbtion from the membrane into the supernatant after Mg^{2+}-ATP treatment), increased between 5 and 30 min and the Mg^{2+}-ATP released particulate PFK activity reached its maximum between 30 and 60 min.

complex comprised of catalytic (40 kDa) and regulatory (PFK isoform) polypeptides [1,2,15]. Since the regulatory polypeptide of the calcium-independent PLA_2 catalytic complex is an isoform of PFK, we hypothesized that the translocation of PFK during the ischemic process could facilitate the delivery of the 40 kDa catalytic entity to specific membrane compartments which serve as substrates for calcium-independent PLA_2. Herein we demonstrate that both PFK protein mass and activity are translocated from the cytosol to a membrane-associated compartment with a time course that was similar to the translocation of the calcium-independent PLA_2 activity during the evolution of ischemic injury [3,4]. Furthermore, the reversible translocation of PFK mass and activity from the particulate to soluble fractions after reperfusion parallels the attenuation of membrane-associated PLA_2 activity induced by reperfusion of ischemic myocardium. Thus, through the direct physical interaction of key components in sugar and lipid metabolism (i.e. PFK and PLA_2), glycolysis and phospholipolysis can be coordinately regulated utilizing the chemical sensory machinery inherent in the structure of PFK.

Collectively, these results demonstrate the rapid and reversible translocation of PFK from a soluble to a particulate fraction during ischemia that follows a temporal course which is indistinguishable from that of the translocation of calcium-independent PLA_2 activity. While the chemical modification(s) of PFK and/or PLA_2 structure underlying the translocation of the PLA_2 catalytic complex remain to be precisely defined, these findings further substantiate the fundamental interrelation-

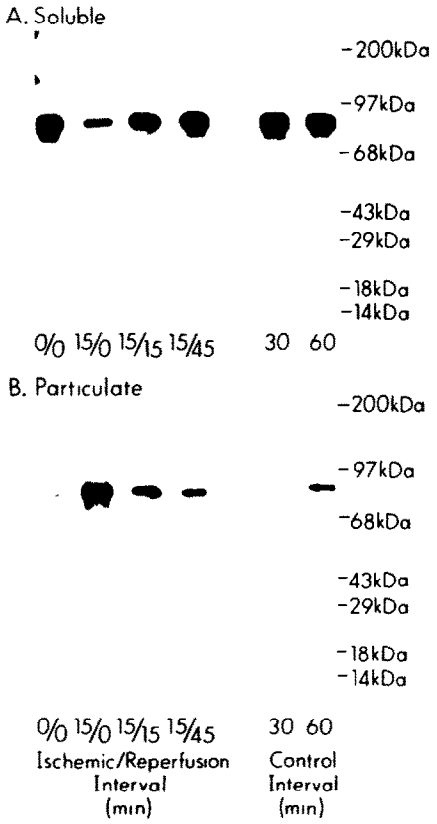

Fig. 3. Rabbit hearts were perfused utilizing a Langendorff model as described in section 2. After a 10 min equilibration period, hearts were rendered globally ischemic for 0 or 15 min intervals without reperfusion (panel A and B; 0/0, 15/0), or rendered globally ischemic for 15 min followed by 15 or 45 min period of reperfusion (panel A and B; 15/15 and 15/45, respectively). Control hearts were perfused for 30 or 60 min at normal flow rates (panel A and B; 30 and 60 min, respectively). After the indicated periods of perfusion and/or reperfusion, the 24,000 × g_{max} supernatant and pellets were solubilized in SDS buffer and subjected to Western blot analysis utilizing affinity purified anti-PFK-antibody. To facilitate direct comparisons, pellets from centrifugation were resuspended in a volume of homogenization buffer equal to that from which they were derived as described in section 2. Panel A demonstrates the decrease in the 85 kDa immunoreactive protein band in the supernatant after 15 min of ischemia without reperfusion (panel A; 0/0, 15/0). After 15 min of ischemia followed by either 15 or 45 min of reperfusion, the 85 kDa immunoreactive protein band increased in intensity (panel A, 15/15, 15/45). Panel B demonstrates an increase in the 85 kDa immunoreactive protein band with 15 min of ischemia without reperfusion (panel B; 0/0, 15/0). With either 15 or 45 min of reperfusion, the 85 kDa immunoreactive protein band decreases in intensity (panel B; 15/15, 15/45).

ship between alterations in phospholipolysis and glycolysis in ischemic myocardium.

Acknowledgements: This research was supported by NIH Grant 34839.

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